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# **Similarity Between Man and Laboratory Animals in Regional Pulmonary Deposition of Ozone**

**(U.S.) Health Effects Research Lab, Research Triangle Park, NC**

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## Similarity between Man and Laboratory Animals in Regional Pulmonary Deposition of Ozone<sup>1</sup>

FREDERICK J. MILLER, DANIEL B. MENZEL,\* AND DAVID L. COFFIN

*Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, North Carolina 27711, and \* Division of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710*

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Predicted pulmonary ozone ( $O_3$ ) dose curves obtained by model analysis of the transport and removal of  $O_3$  in the lungs of guinea pigs, rabbits, and man indicate that a general similarity exists among these species in the shapes of the dose curves. An overview of the major features of the lower airway mathematical model used is presented. This model predicts that the respiratory bronchioles receive the maximum  $O_3$  dose. For exposures corresponding to tracheal  $O_3$  concentrations greater than  $100 \mu g/m^3$  (0.05 ppm), the predicted respiratory bronchiolar dose for rabbits was found to be twice that for guinea pigs and 80% of that for man. Sensitivity analyses are presented for model parameters relating to the treatment of the chemical reactions of  $O_3$  with the mucous layer. The role of tidal volume in the determination of pulmonary uptake of  $O_3$  in man is examined. The consistency and similarity of the dose curves for the three species lend strong support to the validity of extrapolating to man the results obtained on animals exposed to  $O_3$ .

### INTRODUCTION

The assessment of adverse human health effects associated with exposure to environmental air pollutants involves the toxicological evaluation of results obtained from epidemiologic, clinical, and animal studies. One of the strong points of epidemiologic and clinical studies is that the information obtained is directly related to the human experience. Also, subgroups of the general population can be selected for study which correspond to sensitive individuals. Likewise, industrial workers represent a group for study who are routinely exposed to pollutant levels significantly greater than the levels found in the ambient air. Inability to control exposure levels, the mobility of the population, and difficulty in accurately determining previous exposure history and important concomitant variables are uncertainties in epidemiologic studies.

While exposure levels in human clinical trials can be adequately controlled, the types of biological parameters which can be studied must usually be limited to those for which noninvasive measurement techniques are available. As a result of these limitations, and others, the vast majority of toxicological studies are conducted using laboratory animals. In contrast to clinical studies, animal experimentation allows the researcher the choice of a wide range of concentrations, exposure regimens, animal species, chemical agents, and biological parameters. From these studies, the environmental toxicologist must make judgments concerning

<sup>1</sup> The work described in this paper has been taken, in part, from a thesis submitted by F. J. Miller to the faculty of North Carolina State University, Raleigh, N.C., in partial fulfillment of the requirements for the Ph.D. degree.

the validity of extrapolating the results to man. In addressing the likelihood that the animals studied mimic the human response, an important consideration must be to estimate the effective dose delivered to the target organ. Differences between man and experimental animals in the ratio of effective dose to exposure concentration must also be considered.

To achieve these objectives, greater emphasis must be placed upon developing mathematical models for pollutant target organs. These models should incorporate species anatomical differences and physiochemical properties of the pollutant as parameters and should be based upon the physical laws which govern transport and removal of the pollutant. McJilton *et al.* (1972) have proposed a model for predicting the uptake of ozone (O<sub>3</sub>) in the lung. Since chemical reactions of O<sub>3</sub> with components of the mucous layer were not included, their model underestimates conducting airway uptake of O<sub>3</sub> and overestimates respiratory airway uptake. Thus, the model of McJilton *et al.* is more useful for determining the uptake of nonreactive gases than it is for estimating the uptake of O<sub>3</sub>.

An approach for evaluating O<sub>3</sub> toxicity has been presented which empirically accounts for nasopharyngeal removal of O<sub>3</sub> and mathematically models the chemical reactions associated with the transport and removal of O<sub>3</sub> in pulmonary airways (Miller, 1977). This model has been used to examine the similarity in regional pulmonary deposition of O<sub>3</sub> between man and laboratory animals.

#### MATERIALS AND METHODS

##### *Transport Equation for Ozone in a Lower Airway Model*

Since a detailed account of the mathematical model used in this study is presented elsewhere (Miller, 1977), only a summary of the major features of the model will be given here. The transport and removal of O<sub>3</sub> in the lung was studied using the binary convective-diffusion equation

$$\frac{\partial \bar{C}}{\partial t} + \bar{U}_x \frac{\partial \bar{C}}{\partial x} = (D_{\text{mol}} + D_{\text{ed}}) \left( \frac{\partial^2 \bar{C}}{\partial r^2} + \frac{1}{r} \frac{\partial \bar{C}}{\partial r} + \frac{\partial^2 \bar{C}}{\partial x^2} \right) + \bar{S}^*, \quad (1)$$

where  $\bar{C}$ ,  $\bar{U}_x$ , and  $\bar{S}^*$  represent species-averaged population concentration, velocity, and source term values, respectively, in a given airway at a specified location and time. In Eq. (1),  $x$  and  $r$  refer to distance in the axial and radial directions, respectively,  $t$  equals time, the molecular diffusion coefficient of O<sub>3</sub> in air is  $D_{\text{mol}}$ , and  $D_{\text{ed}}$  represents the diffusion coefficient associated with the phenomenon of eddy dispersion. Simply stated, Eq. (1) asserts that the transport and removal of O<sub>3</sub> in the lung is a function of convection, axial and radial diffusion, and chemical reactions.

The amount of O<sub>3</sub> acting on a given level of the lung is reflected by the airway lumen concentration at that level. Ozone does not immediately strike the airway wall; instead, it must first come in contact with the mucous or surfactant layer lining the airway, depending upon which level of the lung the gas has reached. Chemical reactions with components in these layers may occur, thereby increasing the total absorption of O<sub>3</sub>, but may also reduce the amount of O<sub>3</sub> reaching the tissue. Thus, it is clear that the biochemical compositions of the mucous and

surfactant layers dictate the manner in which chemical reactions should be treated.

#### *Biochemical Composition of Mucus and Surfactant*

Clements and co-workers (1970) showed that, among various vertebrate species, the amount of surfactant correlates well with the amount of dipalmitoyl lecithin (DPL) in lung parenchyma and with alveolar surface area. DPL constitutes 90 to 95% of the recoverable lipid in fractional rabbit alveolar lavage fluid (Balis *et al.*, 1971; Hurst *et al.*, 1973). Hence, the alveoli are normally lined with saturated lecithin and are mostly free of other lipids, protein, and carbohydrate.

The lipid fraction in mucus from one asthmatic patient has been extensively characterized by Lewis (1971). Since the same qualitative lipid composition was observed in patients with chronic bronchitis, Lewis concluded that the lipid composition of asthmatic mucus is typical of bronchial mucus. Potter *et al.* (1963) determined the non-water constituents per 100 g of human pulmonary secretions as: 1.13 g of ash, 0.03 g of DNA, 0.95 g of carbohydrate, 1.00 g of protein, and 0.84 g of lipid. To date, detailed biochemical analyses have not been performed on the protein and carbohydrate components of bronchial mucus.

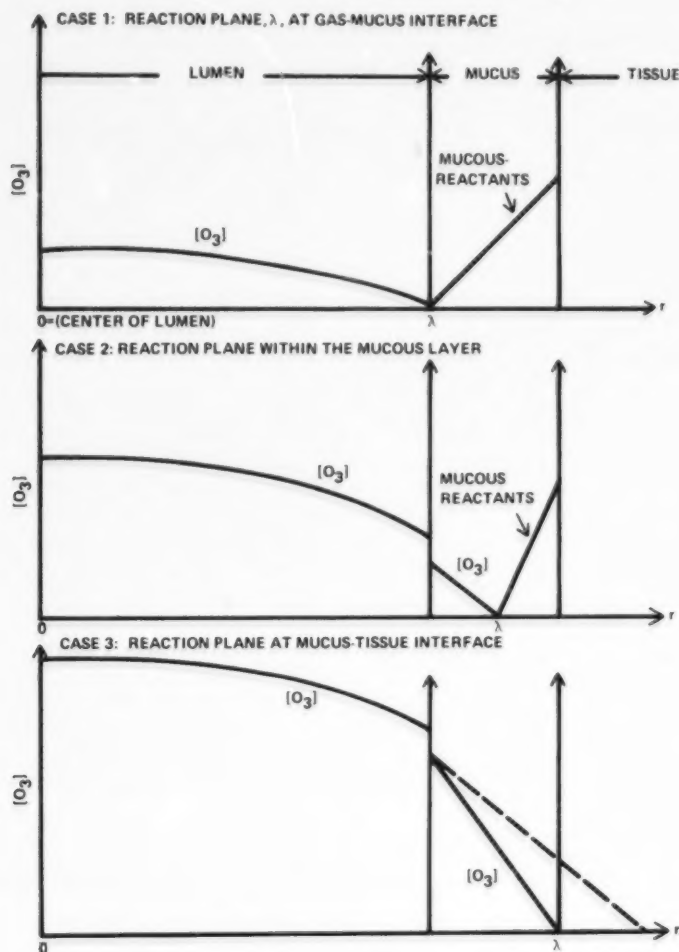
#### *Model Treatment of Chemical Reactions*

Recently, Menzel (1976) reviewed the chemical mechanisms of action of  $O_3$  in biological systems. Olefins are especially susceptible to oxidation by  $O_3$ . The reaction of  $O_3$  with olefins likely involves an initial reaction with the olefin to form the zwitterion (Criegee, 1962), and possibly ozonide, and then a reaction involving the peroxidation of the remaining unreacted olefins. Mudd *et al.* (1969) have determined the order of susceptibility of amino acids in aqueous solutions to oxidation by  $O_3$ .

A direct attack of  $O_3$  upon the double bonds of the fatty acid is involved in the initiation of the reaction of  $O_3$  with olefins. This reaction is irreversible (Menzel, 1976). Moreover, since the reactions of  $O_3$  with amines and olefins are diffusion-controlled (Criegee, 1962; Menzel, 1976), they can be characterized by an instantaneous-reaction regime.

Basic to the instantaneous-reaction scheme is the concept that the absorbing component, in this instance  $O_3$ , and the liquid-phase reactant cannot coexist in the same region of the liquid. Hence, the liquid can be considered as essentially being comprised of two layers (Astarita, 1967). In the first layer, diffusion of  $O_3$  occurs, while diffusion of the liquid-phase reactant takes place in the second layer. The boundary between the two layers is defined to be  $\lambda$ , the reaction plane. At the reaction plane the concentrations of both  $O_3$  and the liquid-phase reactant are zero.

It can be shown that three cases are needed to characterize removal of  $O_3$  via chemical reactions in airways lined by mucus (Miller, 1977). These cases are a function of the lumen  $O_3$  concentration and are depicted in Fig. 1. As the lumen  $O_3$  concentration in a given airway rises and falls during the breathing cycle, the reaction plane may be established anywhere from the gas-mucus interface (case 1) to a position equivalent to  $O_3$  penetrating to the tissue (case 3). The dashed line in case 3 indicates where the reaction plane would be if the mucous layer were

FIG. 1.  $O_3$ -mucus reaction cases.

deep enough. However, the solid line is imposed by the restriction that  $O_3$  is instantaneously converted by chemical reactions when it reaches the tissue.

In the lung, the conducting airways are lined with mucus, while the transitory airways (respiratory bronchioles) and respiratory regions are lined with surfactant. Since surfactant consists almost entirely of saturated lecithin, no chemical reactions of  $O_3$  with the surfactant layer were assumed. Hence, chemical reactions with the layer lining the airways were considered only for conducting airways.

The data of Lewis (1971) were used to estimate the number of mole equivalents represented by the double bonds of the unsaturated fatty acids of the major lipid components of human bronchial mucus (Table 1). The amino acids found in

TABLE I  
MOLE EQUIVALENT CONCENTRATIONS OF O<sub>3</sub> REACTANTS PRESENT  
IN HUMAN BRONCHIAL MUCUS

| Source <sup>a</sup>                                     | Fatty acid | Weight percent | moles/cm <sup>3</sup> × 10 <sup>7</sup> | mole equivalents/cm <sup>3</sup> × 10 <sup>7</sup> |
|---------------------------------------------------------|------------|----------------|-----------------------------------------|----------------------------------------------------|
| Free fatty acids<br>(18.1%) <sup>b</sup>                | 16:1       | 4.0            | 0.450                                   | 0.450                                              |
|                                                         | 18:1       | 30.7           | 3.100                                   | 3.100                                              |
|                                                         | 18:2       | 8.0            | 0.810                                   | 1.620                                              |
| Triglycerides<br>(39.0%)                                | 16:1       | 0.6            | 0.138                                   | 0.138                                              |
|                                                         | 18:1       | 8.0            | 1.660                                   | 1.660                                              |
|                                                         | 18:2       | 1.1            | 0.230                                   | 0.460                                              |
|                                                         | 20:2       | 1.8            | 0.344                                   | 0.688                                              |
| Cholesterol esters<br>(2.8%)                            | 16:1       | 8.0            | 0.056                                   | 0.056                                              |
|                                                         | 18:1       | 26.4           | 0.178                                   | 0.178                                              |
|                                                         | 18:2       | 37.7           | 0.255                                   | 0.510                                              |
|                                                         | 18:3       | 0.9            | 0.006                                   | 0.018                                              |
| Phosphatidyl choline <sup>c</sup><br>(11.0%)            | 16:1       | 1.2            | 0.053                                   | 0.053                                              |
|                                                         | 18:1       | 22.2           | 0.972                                   | 0.972                                              |
|                                                         | 18:2       | 9.2            | 0.403                                   | 0.806                                              |
| Phosphatidyl ethanolamine <sup>c,d</sup><br>(5.0%)      | 16:1       | 1.2            | 0.025                                   | 0.025                                              |
|                                                         | 18:1       | 22.2           | 0.468                                   | 0.468                                              |
|                                                         | 18:2       | 9.2            | 0.194                                   | 0.388                                              |
| Lysophosphatidyl choline and sphingomyelin <sup>d</sup> | 16:1       | 1.2            | 0.011                                   | 0.011                                              |
|                                                         | 18:1       | 22.2           | 0.205                                   | 0.205                                              |
|                                                         | 18:2       | 9.2            | 0.085                                   | 0.170                                              |
| Histidine                                               |            |                |                                         | 5.800                                              |
| Total                                                   |            |                |                                         | 17.776                                             |

<sup>a</sup> All data derived from Lewis (1971), except for histidine (Levine *et al.*, 1969; Marshall, 1972; Mudd *et al.*, 1969; Potter *et al.*, 1963).

<sup>b</sup> Percentage of total lipid weight.

<sup>c</sup> The current study assumes both fatty acids on this phospholipid are the same.

<sup>d</sup> The current study assumes the weight percentage of this phospholipid is the same as that of phosphatidylcholine.

human parotid glycoproteins were used to approximate the amino acid compositions of glycoproteins in bronchial mucus (Levine *et al.*, 1969). Of the amino acids present, only histidine was susceptible to oxidation by O<sub>3</sub> (Mudd *et al.*, 1969). For histidine, the number of mole equivalents of O<sub>3</sub> given in Table I can be determined using Table 6 (for the moles of histidine per mole of glycoprotein) from Marshall (1972) and the estimate of 1.0 g of protein/g of mucus from Potter *et al.* (1963). Hence, the concentration of O<sub>3</sub> reactants present in mucus are collectively assessed by the data in Table I.

#### Structure of the Model

The model formulation requires extensive use of lung morphometric data. For man, the regularized dichotomously branched lung data of Weibel (1963) were used, while for rabbits and guinea pigs, the morphometric data was based upon the

work of Kliment (1973). Each airway generation or morphometric zone was partitioned to form a sequence of model segments, and a multistep procedure involving mass balance expressions in finite difference form was used to obtain solutions of Eq. (1). Respiration was assumed to be sinusoidal.

Convection was simulated by instantaneously moving a small gas volume (approximately 1/200th of the normal tidal volume) through the airways during each time interval. Within each model segment a new lumen concentration was obtained for this same time interval. Changes in the lumen profile due to axial diffusion were determined next, followed by radial decay, during which radial diffusion accounted for mass transport of O<sub>3</sub> to the fluid layer lining the given model segment, where removal of O<sub>3</sub> from the airway occurs. The lumen concentration was again determined and this entire sequence was repeated for the next time increment. The number of model segments for each species and the use of 200 time steps for numerical analysis were obtained as a result of convergence testing (Miller, 1977).

The joint effects of molecular and eddy diffusivity, ( $D_{mol} + D_{ed}$ ) of Eq. (1), were estimated from a modification of the results of Scherer and co-workers (1975). These investigators determined effective axial diffusivity,  $D_x$ , for laminar flow of a gas in the conducting airways and obtained

$$D_x = D_{mol} (1 + \gamma N_{pe}) \quad (2)$$

where  $\gamma$  equals 1.08 for inspiration and 0.37 for expiration, and  $N_{pe}$  refers to the Peclet number of that region of the airways where  $D_x$  applies. By definition,  $N_{pe} = 2R\bar{U}_x/D_{mol}$ , where  $R$  is the airway radius. The method used to determine  $D_x$  effectively replaced five generations by a single one which had an average axial velocity and cross-sectional area equal to those of the initial generation. However, the procedure of Scherer *et al.* retained the effects of bifurcations, turbulence, and secondary flows.

Since predictions of O<sub>3</sub> absorption in each generation of the tracheobronchial tree were desired in the current model study,  $D_x$  was modified by considering the  $j$ th generation as the focal point of these five-generation simplifications. An average diffusion coefficient was computed in the  $j$ th airway generation at each time step as

$$\begin{aligned} D &= D_{mol} \left( 1 + \frac{\gamma}{5} \sum_{i=j-2}^{j+2} \frac{2R(i)\bar{U}_x(i)}{D_{mol}} \right), \\ &= D_{mol} + \frac{2\gamma}{5} \sum_{i=j-2}^{j+2} R(i)\bar{U}_x(i), \end{aligned} \quad (3)$$

where  $R(i)$  and  $\bar{U}_x(i)$  represent the radius and average axial gas velocity in the  $i$ th generation, respectively. Although replacing ( $D_{mol} + D_{ed}$ ) in Eq. (1) by  $D$  defined in Eq. (3) is not precisely correct, it represents a substantial improvement over previous models, which considered only molecular diffusion in characterizing the effects of diffusion in the airways.

The model structure for radial decay in the conducting airways involves the effective rate of production per unit area, at the airway wall, of the components of mucus with which  $O_3$  will react (Miller, 1977). Since the morphometric data for rabbits and guinea pigs is only available by "zone," while the data for man is given by dichotomously branched generation, "mucous production zones" were formed for humans by considering which generations had drained the same percentage of mucous-lined airway surface area as had a given morphometric zone in rabbits and guinea pigs. Goblet cell density, expressed as number of goblet cells per five high-power microscopic fields, has been shown in rats to decrease between the trachea and distal airways (Reid, 1970). In the present study, the effective rate of production of mucous reaction components per unit area was assumed to decrease linearly, with respect to mucous zone, from a maximum value at the trachea to zero at the beginning of the respiratory bronchioles. The data of Luchtel (1976) were used to assign values for the thickness of the mucous layer.

## RESULTS

Using the model approach described in previous sections, transport of  $O_3$  in the lungs of man, rabbits, and guinea pigs was studied. Subsequent sections illustrate physiological and toxicological comparisons that can be obtained and examine the sensitivity of the results with respect to some of the model parameters.

### *Predicted Dose Curves with Normal Respiration*

Model estimates for the dose of  $O_3$  delivered to the tissue in various generations of the human lung are shown in Fig. 2. Independent of the inhaled tracheal concentration of  $O_3$ , the model predicts that the first generation of respiratory bronchioles (generation 17) receives the maximum dose of  $O_3$ . While the model predicts uptake of  $O_3$  by respiratory airway tissue for all tracheal concentrations studied, penetration of  $O_3$  to the tissue in airways lined by mucus (generation 16 and above) is not predicted for inhaled tracheal concentrations less than  $125 \mu\text{g}/\text{m}^3$  (0.06 ppm). Presumably, at these low exposure levels, the concentration of  $O_3$  reactants and the thickness of the mucous layer combine to deplete all of the  $O_3$  absorbed in the conducting airways. However, as the inhaled tracheal concentration is increased from 250 to  $4000 \mu\text{g}/\text{m}^3$ , the tissue deposition pattern in the conducting airways becomes smoother, increases in magnitude, and includes more airways. Irrespective of tracheal concentration, alveolar  $O_3$  doses (generations 20–23) decline sharply with increasing generation number due in large part to the tremendous increase in surface area.

Corresponding to Fig. 2, the relative contributions of mucus, conducting airway tissue, and respiratory airway (surfactant-lined) tissue to total uptake are depicted in Fig. 3. As the tracheal concentration of  $O_3$  increases, the percentage uptake of  $O_3$  by mucus declines sharply and assumes an approximately constant value of 7 to 9% for inhaled tracheal  $O_3$  concentrations exceeding  $750 \mu\text{g}/\text{m}^3$  (0.38 ppm). The uptake of  $O_3$  by respiratory tissue is, on the other hand, almost a mirror image of that by mucus. Respiratory airway uptake increases rapidly as the concentration of inhaled  $O_3$  increases and plateaus at about 46%. The model predicts that the contribution of conducting airway tissue doses to the total uptake of  $O_3$  is slight (less than 8% for all concentrations).

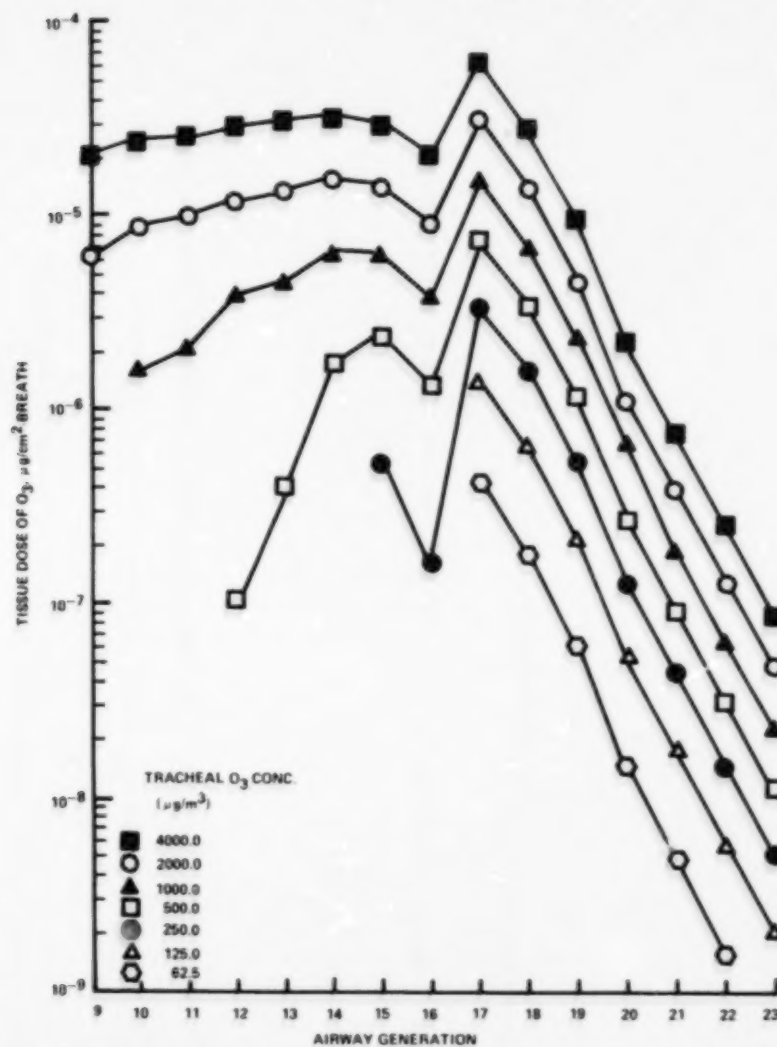


FIG. 2. Predicted tissue deposition of ozone for various regions of the human lung. Model analyses used a tidal volume of 500 ml and a respiratory frequency of 15 breaths/min.

Tissue dose patterns of  $O_3$  in the lungs of rabbits and guinea pigs are presented in Figs. 4 and 5, respectively. As for man, the respiratory bronchioles (model zone 6) are predicted to receive the maximum dose in both animal species. Although several human airway generations may correspond to a morphometric zone in rabbits and guinea pigs, a general similarity is evident between the three species in the shapes of the predicted dose curves. The predicted doses for alveolar ducts



FIG. 3. Uptake of ozone by mucus and airway walls as a function of tracheal concentration of  $O_3$  in the human lung. Model conditions were the same as for Fig. 2.

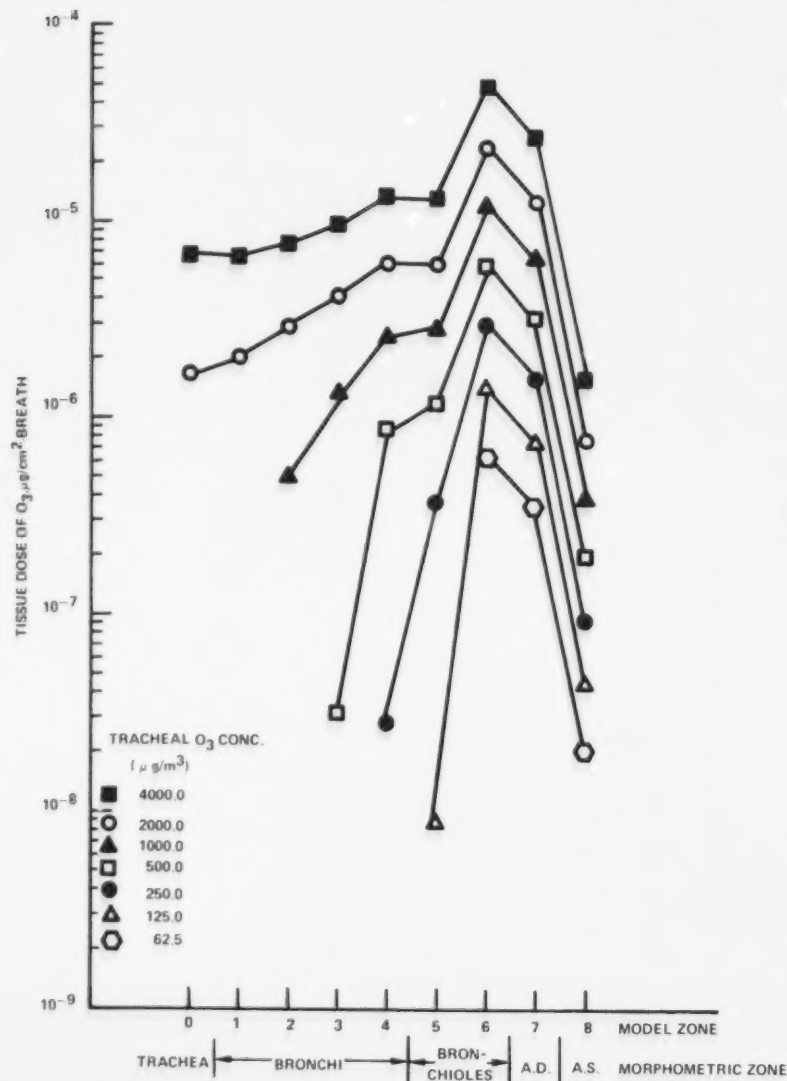


FIG. 4. Predicted tissue deposition of ozone for various regions of the rabbit lung. Model analyses used a tidal volume of 21 ml and a respiratory frequency of 50 breaths/min.

and sacs exhibit a greater difference in rabbits, as compared to guinea pigs. Also, Figs. 4 and 5 show that with exposure to a tracheal  $O_3$  level of  $1000 \mu g/m^3$  (0.51 ppm), penetration of  $O_3$  to the tissue occurs in the trachea of guinea pigs, but not until the second-order bronchi are reached in rabbits.

The relationship between respiratory bronchiolar dose and the inhaled tracheal

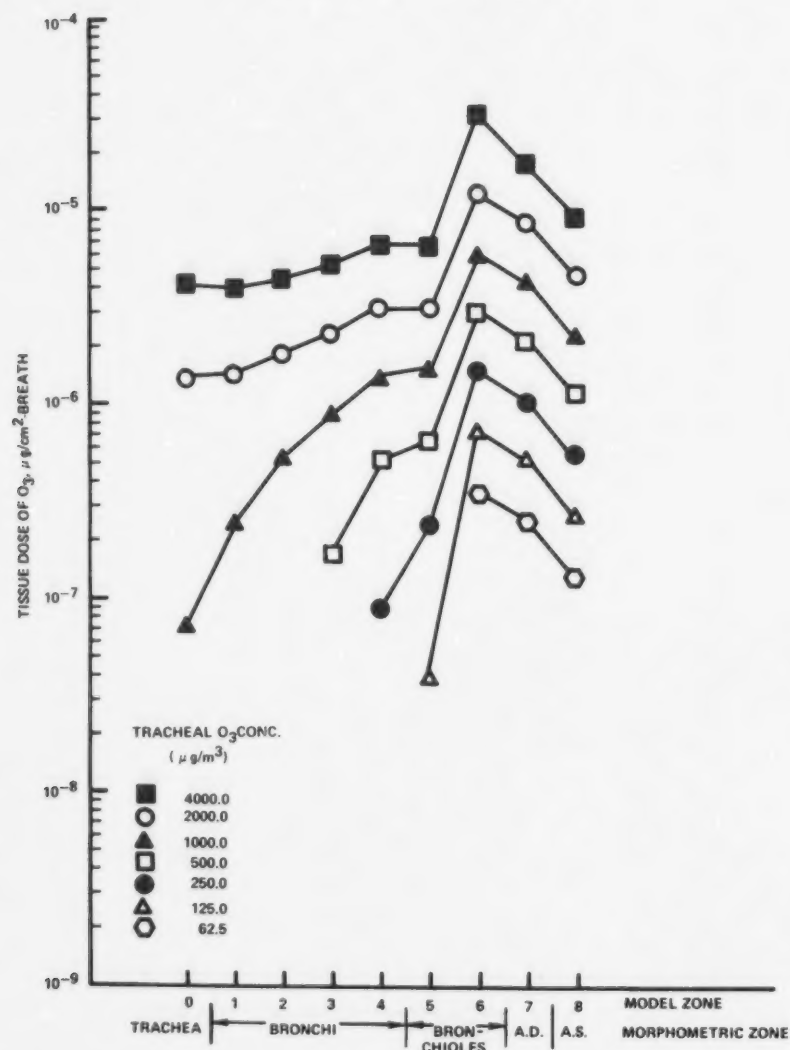


FIG. 5. Predicted tissue deposition of ozone for various regions of the guinea pig lung. Model analyses used a tidal volume of 1.8 ml and a respiratory frequency of 90 breaths/min.

$O_3$  concentration is given in Fig. 6. For tracheal concentrations exceeding  $100 \mu g$  of  $O_3/m^3$  (0.05 ppm  $O_3$ ), this relationship is linear on a logarithmic scale for all three species. From regression analysis applied to the data in Fig. 6 for tracheal  $O_3$  concentrations between 100 and  $4000 \mu g/m^3$  (0.05 and 2.04 ppm), the respiratory bronchiolar dose of  $O_3$  over this range is proportional to the tracheal concentration raised to the 1.0 power for guinea pigs and rabbits and to the 1.07 power for man.

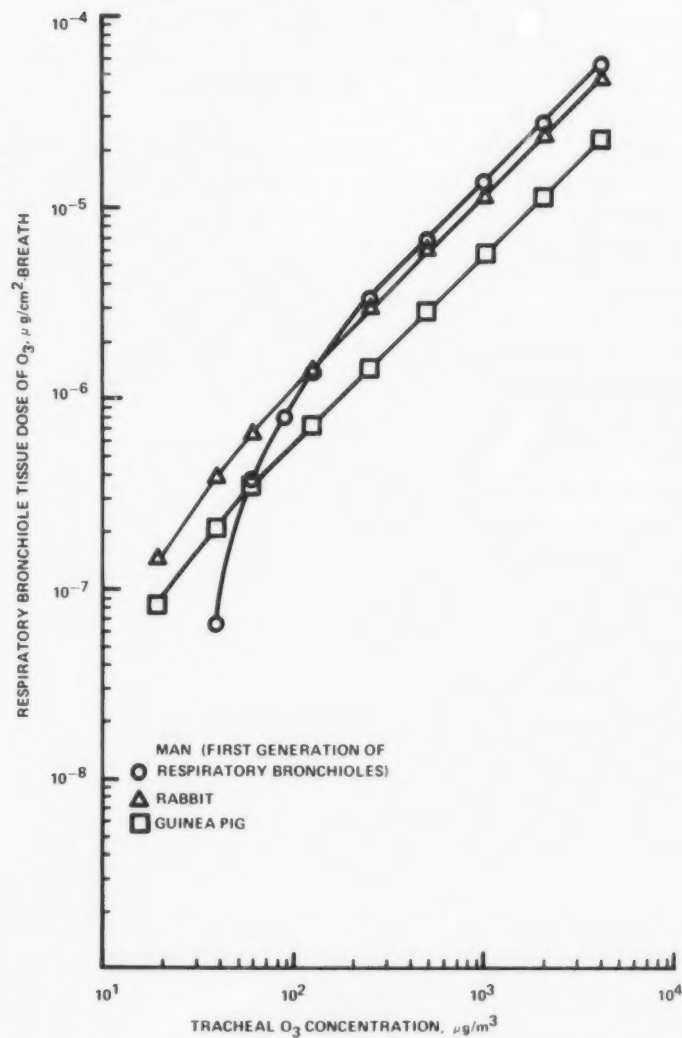


FIG. 6. Relationship between respiratory bronchiolar dose and concentration of ozone in the trachea of guinea pigs, rabbits, and man. Model analyses for man used a tidal volume ( $V_T$ ) of 500 ml and a respiratory frequency ( $f$ ) of 15 breaths/min. For rabbits  $V_T = 21$  ml and  $f = 50$  breaths/min, while for guinea pigs  $V_T = 1.8$  ml and  $f = 90$  breaths/min.

For any given tracheal  $O_3$  concentration greater than  $100 \mu g/m^3$ , the predicted respiratory bronchiolar dose for rabbits is twice that for guinea pigs and is 80% of that for man.

With pollutant exposures corresponding to less than  $100 \mu g$  of  $O_3/m^3$  at the trachea, the predicted respiratory bronchiolar dose curve in man is markedly

different than the curve for rabbits and for guinea pigs (Fig. 6). As the tracheal  $O_3$  concentration decreases from  $100 \mu\text{g}/\text{m}^3$ , there is a more gradual decline in the dose curve for rabbits and guinea pigs than there is in the dose curve for man.

#### Model Sensitivity Analyses

Basic to the model structure for radial decay in the conducting airways is the treatment of the production of the components of mucus which will react with  $O_3$ . Obtaining estimates of these production rates involved  $\bar{K}_0$ , the average rate of production per unit area of reaction components (Miller, 1977). The question arises as to the sensitivity of the model estimated doses to the value of  $\bar{K}_0$ .

Model analyses were conducted in which  $\bar{K}_0$  was varied between  $100\times$  smaller and  $100\times$  larger than the value of  $\bar{K}_0$  (standard) used to obtain the figures presented previously. It should be noted that decreasing  $\bar{K}_0$  10-fold, for example, is equivalent to keeping the velocity of the mucus the same and having 1/10th the concentration that was used in the standard model analyses of the components of mucus which will react with  $O_3$ . The predicted respiratory bronchiolar dose in rabbits as a function of  $\bar{K}_0$  is shown in Fig. 7. The tracheal  $O_3$  concentration studied,  $325 \mu\text{g}/\text{m}^3$  (0.166ppm), corresponds to an inhaled ambient concentration of  $490 \mu\text{g}/\text{m}^3$  (0.25 ppm), after accounting for nasopharyngeal removal of  $O_3$  (Miller, 1977). Alveolar region effects of  $O_3$  have been shown in rabbits exposed to this level (Hurst *et al.*, 1970) and provide a means of evaluating the biological implications of different predicted doses.

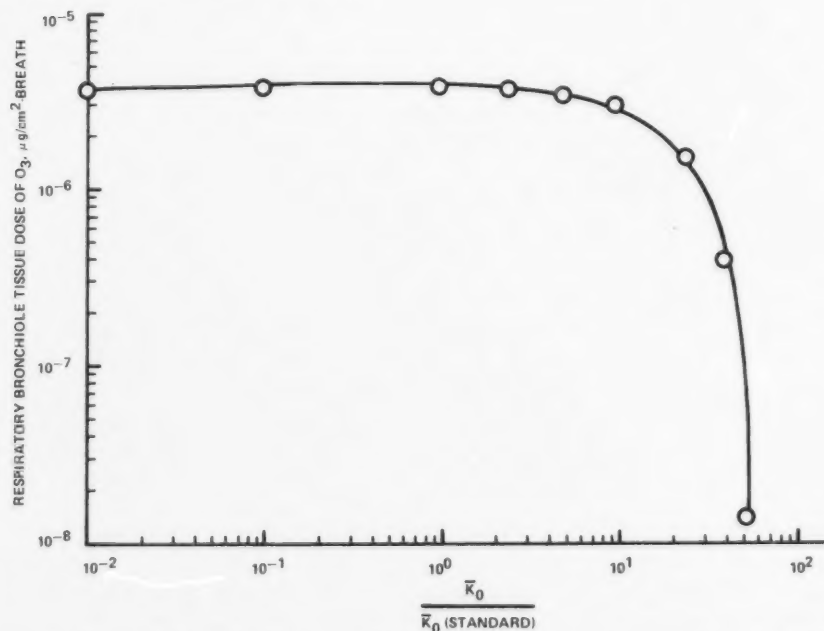


FIG. 7. Sensitivity of respiratory bronchiolar ozone dose to the average current of mucous reaction components ( $325 \mu\text{g}$  of  $O_3/\text{m}^3$  tracheal concentration in rabbits)

If  $\bar{K}_0$  (standard) represents up to a 100-fold overestimation of the correct value, there is no difference in the predicted respiratory bronchiolar dose (Fig. 7). Also, a 10-fold underestimation of the correct value of  $\bar{K}_0$  yields no appreciable change in respiratory bronchiolar dose. Only if the correct value has been underestimated at least 25-fold, does the model predict significantly different O<sub>3</sub> doses for the respiratory bronchioles.

Although not presented, even with a 25- to 100-fold underestimation of  $\bar{K}_0$ , the general shape of the dose curve for the respiratory regions is still the same. Only the relative location on the dose-axis and the shape of the conducting airway portion of the curve are changed. In the conducting airways, decreasing  $\bar{K}_0$  allows penetration of O<sub>3</sub> to the tissue higher in the respiratory tract, but the magnitudes of the doses obtained still do not approach those predicted for the respiratory bronchioles. Increasing  $\bar{K}_0$  results in less penetration of O<sub>3</sub> to conducting airway tissue.

The dose of O<sub>3</sub> delivered to conducting airway tissue can also be decreased by increasing the thickness of the mucous layer. A 25% increase in the thickness of the mucous layer was not sufficient to alter the predicted dose of O<sub>3</sub> to the respiratory regions in man. However, in the conducting airways the dose dropped slightly for higher tracheal O<sub>3</sub> concentrations, but dropped significantly at the lower concentrations studied. There may also be a shift in the prediction of the earliest airway generation in which O<sub>3</sub> penetrates to the tissue.

#### *Relationship of Tidal Volume to Deposition*

As an example of the respiratory parameters which can be studied using the model, the percentage uptake of O<sub>3</sub> in the human lung by mucus and airway walls, as a function of tidal volume, is shown in Fig. 8. An inspiratory time of 2.0 sec was used for tidal volumes of 1250 ml or less, while increased respiratory rates associated with tidal volumes of 1500 and 1750 ml were accounted for by using inspiratory times of 1.64 and 1.48 sec, respectively (Vander *et al.*, 1970). Figure 8 shows that for a tracheal concentration of 750  $\mu\text{g}$  of O<sub>3</sub>/m<sup>3</sup> (0.38 ppm), as tidal volume increases over the range from 500 to 1750 ml, the percentage uptake of O<sub>3</sub> by respiratory airway tissue doubles from 46 to 92%. There is a gradual decline in the percentage uptake of O<sub>3</sub> by mucus and conducting airway tissue as tidal volume increases. Over the range of tidal volumes studied with this tracheal concentration (750  $\mu\text{g}$  of O<sub>3</sub>/m<sup>3</sup>), the percentage uptake of O<sub>3</sub> by mucus and by conducting airway tissue did not exceed 11 and 5%, respectively.

Table 2 gives additional data concerning the effects of tidal volume on respiratory airway uptake of O<sub>3</sub>. There is a plateau in the total O<sub>3</sub> removed in the first generation of respiratory bronchioles as tidal volume increases. However, when considering the uptake of all respiratory areas, the plateau is not present, indicating that alveolar region O<sub>3</sub> doses continue to increase as tidal volume increases. The model predicts a 7-fold increase in the quantity of O<sub>3</sub> removed in the respiratory regions of the lung for a 3.5-fold increase in tidal volume (Table 2).

#### DISCUSSION

The O<sub>3</sub> dose curves obtained in this study, by model analysis of the transport and removal of O<sub>3</sub> in the lungs of guinea pigs, rabbits, and man, indicate that the respiratory bronchioles receive the maximum dose. The model results are in good

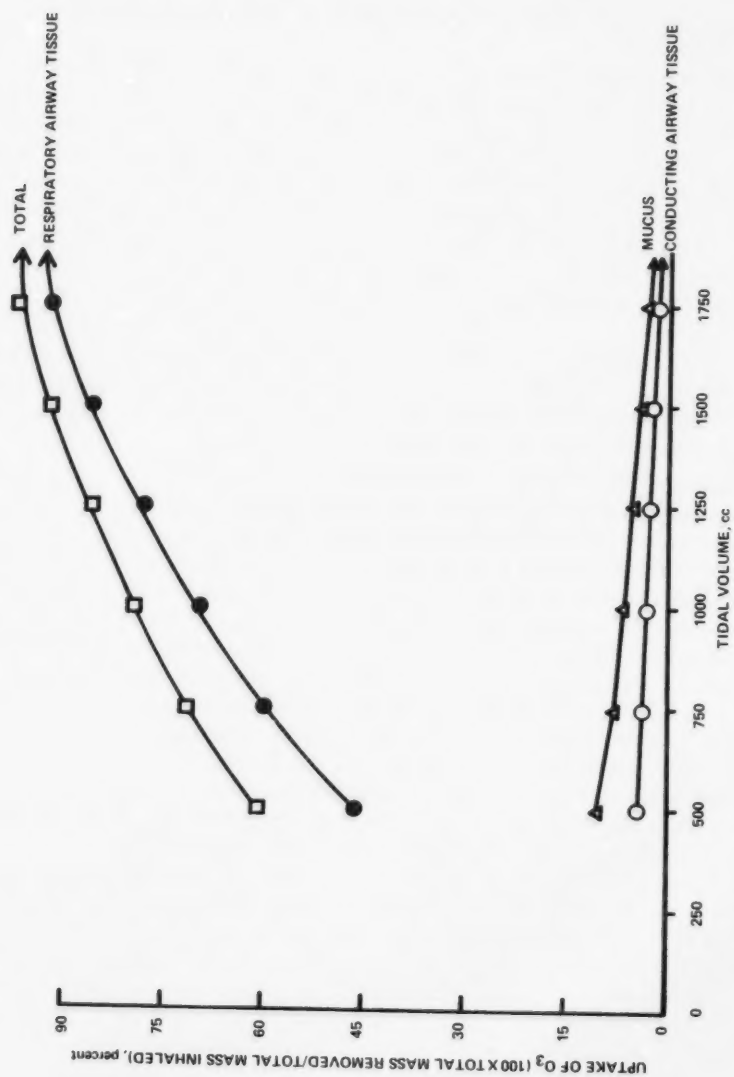


FIG. 8. Uptake of ozone by mucus and airway walls as a function of tidal volume ( $750 \mu\text{g}/\text{m}^3$  tracheal concentration in man).

TABLE 2  
RESPIRATORY AIRWAY UPTAKE OF O<sub>3</sub> IN MAN FOR VARIOUS TIDAL VOLUMES (V<sub>T</sub>)<sup>a</sup>

| V <sub>T</sub><br>(ml) | Total O <sub>3</sub> | Respiratory bronchiole <sup>b</sup> |                                        | All respiratory regions |                          |
|------------------------|----------------------|-------------------------------------|----------------------------------------|-------------------------|--------------------------|
|                        | inhaled<br>(μg)      | Uptake<br>(μg)                      | Uptake/uptake<br>(V <sub>T</sub> =500) | Uptake<br>(μg)          | Uptake/uptake<br>(V=500) |
| 500                    | 0.375                | 0.033                               | 1.00                                   | 0.173                   | 1.00                     |
| 750                    | 0.5625               | 0.052                               | 1.58                                   | 0.336                   | 1.94                     |
| 1000                   | 0.75                 | 0.067                               | 2.03                                   | 0.522                   | 3.02                     |
| 1250                   | 0.9375               | 0.079                               | 2.39                                   | 0.728                   | 4.21                     |
| 1500                   | 1.125                | 0.080                               | 2.42                                   | 0.970                   | 5.61                     |
| 1750                   | 1.3125               | 0.081                               | 2.45                                   | 1.210                   | 6.99                     |

<sup>a</sup> 750 μg of O<sub>3</sub>/m<sup>3</sup> tracheal concentration.

<sup>b</sup> First generation of respiratory bronchioles.

agreement with experimental findings in various animal species. Stephens *et al.* (1973) showed that in rats the Type 1 epithelial cells lining the alveoli immediately beyond the terminal bronchioles are particularly sensitive to O<sub>3</sub> exposure. These cells were severely damaged or removed from large areas with as little as 2 hr of exposure to 980 μg of O<sub>3</sub>/m<sup>3</sup> (0.5 ppm). After 6 to 10 hr of exposure, necrotic ciliated cells were observed free in the lumen of the terminal bronchiole.

In subsequent studies designed to analyze ultrastructural changes and the sequence of events leading to destruction of the Type 1 cell, Stephens *et al.* (1974) reported that, as the distance from the point of maximal effect increased, the observed damage rapidly decreased, until only mild mitochondrial and cytoplasmic swelling were observed. These investigators concluded that the threshold exposure level in rats is below 980 μg of O<sub>3</sub>/m<sup>3</sup> for 2 hr.

In primates exposed to O<sub>3</sub> 8 hr/day for 7 days, Dungworth *et al.* (1975) reported that the most obvious and consistent pulmonary lesions were found in respiratory bronchioles. With exposure to 392 μg of O<sub>3</sub>/m<sup>3</sup> (0.2 ppm), the lesion was primarily observed in the proximal generation of respiratory bronchioles. The damage extended to include some proximal portions of alveolar ducts when the O<sub>3</sub> exposure level was 1568 μg/m<sup>3</sup> (0.8 ppm). Emphysematous lesions, characterized by the apparent loss of alveolar walls and the clubbing of their ruptured extremities, and disruption of the epithelial lining in some bronchioles were found in rabbits exposed to 784 μg of O<sub>3</sub>/m<sup>3</sup> (0.4 ppm) over a 10-month period (P'an *et al.*, 1972). Also, a significant mural thickening of small pulmonary arteries was observed.

There is also evidence from human clinical studies that the respiratory bronchioles are the primary target for O<sub>3</sub>. Bates *et al.* (1972) detected significant changes in the following pulmonary function parameters, as a result of 2 hr of exposure to 1470 μg of O<sub>3</sub>/m<sup>3</sup> (0.75 ppm): (1) a reduction of the maximum static elastic recoil pressure of the lung, (2) an increase in pulmonary resistance and (3) a fall in flow rate at 50% of the actual vital capacity. Even more pronounced changes were observed on various pulmonary function parameters when subjects alternated 15-min periods of exercise and rest.

The level of exercise employed was sufficient to increase minute ventilation from 8–10 to 18–20 liters/min. If nasopharyngeal removal of O<sub>3</sub> in man should

prove to be similar to that for rabbits and guinea pigs, the exposure level used by Bates *et al.* (1972) would correspond to a tracheal concentration of  $O_3$  of approximately  $750 \mu\text{g}/\text{m}^3$  (Miller, 1977). With this concentration and a tidal volume of 1250 ml (sufficient to increase minute ventilation to 18–20 liters/min), the model doses given in Table 2 predict that the first generation of respiratory bronchioles receive a 2.39-fold increase in dose, compared to normal respiration. Also, the total  $O_3$  removed by all respiratory regions is more than 4-fold greater.

One should be cognizant that the pulmonary doses presented in this paper were not experimentally determined, but rather were obtained using a mathematical model which incorporates morphometric and physiochemical data in an analysis of the convective-diffusion equation governing transport and removal of  $O_3$  in the lung. Species differences in nasopharyngeal removal of  $O_3$  must be taken into account before making comparisons on the effective dose of  $O_3$  to various regions of the lung. Additional information in such areas as thickness of the mucous layer in man, production rates of reaction components in mucus, and effective axial diffusivities in guinea pigs and rabbits are needed before a more precise prediction can be made of absolute differences in dose among species. However, based upon the model sensitivity analyses that were performed, considerable differences in the data base are needed before the model predicted doses would be affected. Even then, only the relative location on the dose-axis and the shape of the conducting airways portion of the dose curves would be changed.

Previously, Kliment (1973) showed a high degree of similarity of aerosol particle deposition in the lung among guinea pigs, rabbits, rats, and man, suggesting that deposition data may be compared between these species. Of particular importance in the current study is the similarity among guinea pigs, rabbits, and man of the shape of the predicted dose curves associated with exposure to  $O_3$ . With exposures yielding tracheal  $O_3$  concentrations greater than  $100 \mu\text{g}/\text{m}^3$  (0.05 ppm), the predicted respiratory bronchiolar dose for rabbits was found to be twice that for guinea pigs and 80% of that for man. The consistency and similarity of the dose curves for the three species lend strong support to the validity of extrapolating to man the results obtained on animals exposed to  $O_3$ .

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